nature portfolio

Corresponding author(s): Nadja Zeltner

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	LAS X, Gen5 3.05, Axion AxIS Navigator, Axion CardiacMetric Tool, CFX96 Real-time System, CytExpert 2.0
Data analysis	Prism 9, Fiji (ImageJ), CFX Maestro

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data generated or analyzed during this study are included in the manuscript (and its supplementary information files). Individual data points can be accessed upon request with the corresponding author. Bulk RNA-seq data used in this study can be at the National Center for Biotechnology Information Gene Expression Omnibus under accession nos. GSE212255 (day 35 symNs) and GSE253235 (day 10 NC and day 14 symNblast).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were a minimum of 3 biological repeats (for in vitro differentiations, this was defined as independent differentiations started on different days from consecutive splits or from freshly thawed cells) or experimental animals. However, in most experiments more than 3 biological replicates were assessed, mostly when the distribution/variation between the data points was large.
Data exclusions	Data were excluded when the values were out of the range of 2 standard deviation (SD). This criteria was decided before the data was acquired.
Replication	In our previous work (Wu et al., 2020, JoVE) we established checkpoints to assess the quality of individual differentiations. Here, early stage of neuron differentiation (until day 14) was following the published checkpoints. Replicate was abandoned if one or more checkpoints were not passed properly. After assembloid formation, sizes that are smaller than 0.5 mm will be excluded.
Randomization	Randomization was not applicable in our studies.
Blinding	The investigators were not blinded in any experimental setting as blinding was not feasible or possible.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

α-actinin Sigma A7811
αSMA Sigma A5228
c-Cas3 Cell Signaling 9661
cTnT Proteintech 15513-1-AP
MLC-2a Synaptic Systems 311011
MLC-2v Proteintech 10906-1-AP
NFATC1 Proteintech 66963-1-Ig
PRPH Santa Cruz Biotechnology SC-377093/H0112
TH Pel-Freez P40101- 150
Vimentin Abcam ab92547
VMAT2 R&D Systems MAB8327
WT1 Abcam ab89901

Validation

α-actinin reactive to mouse, human, frog, pig, feline, chicken, hamster, canine, bovine, fish, snake, rabbit, sheep, goat, rat, and lizard applicable for WB, IF, and IHC, according to manufacturer
αSMA reactive to human, mouse, rat, chicken, frog, canine, rabbit, guinea pig, goat, bovine, sheep, snake applicable for ARR, ELISA (i), ICC, IF, IHC (p), WB, according to manufacturer
c-Cas3 reactive to human, mouse, rat, monkey applicable for WB, IF, IP, flow and IHC, according to manufacturer
cTnT reactive to Human, Mouse, Rat, Pig applicable for WB, IHC, IF, FC, ELISA, according to manufacturer
MLC-2a reactive to human, mouse, rat applicable for WB, IF, IP, ICC and IHC, according to manufacturer
MLC-2v reactive to Human, Mouse, Rat, Monkey, Zebrafish, Pig applicable for WB, IP, IHC, IF, FC, ELISA, according to manufacturer
NFATC1 reactive to Human, Mouse, Rat, Pig applicable for WB, IP, IHC, IF, ELISA, according to manufacturer
PRPH reactive to mouse, rat and human applicable for WB, IP, IF, IHC-P and ELISA,according to manufacturer
TH reactive to Mammalian, Non-Mammalian applicable for WB, IHC, IF,according to manufacturer
Vimentin reactive to Mouse, Rat, Human, African green monkeyapplicable for Flow Cyt (Intra), ICC/IF, WB, IHC-P, mIHC, according to manufacturer
VMAT2 reactive to human applicable for WB, ELISA, according to manufacturer
WT1 reactive to Mouse, Human applicable for WB, IHC-P, Flow Cyt (Intra), ICC/IF, according to manufacturer

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Fibroblasts were purchased from Coriell, under the catalog numbers S2=GM04899, S3=GM04589, C1=AG02602

Authentication

Authentication of cell lines was described in Zeltner et al., 2016 nature Medicine

Mycoplasma contamination

ll cell line were tested to be negative for mycoplasma contamination every two weeks.

Commonly misidentified lines
(See ICLAC register)

N/A

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A